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REMARKS

At the outset, Applicants wish to thank the Examiner for the telephone interview of September 12, 2002. In the interview, the Examiner agreed to consider arguments over the written description rejection of claim 10 in view of a proposed amendment requiring the claimed DNA polymerase to have a mutation in the Region II consensus sequence DXXSLYPSII and description of a representative number of species meeting that requirement in the specification. The Examiner also agreed to consider further arguments regarding the abbreviation of the deoxynucleotide thymidine triphosphate, and agreed that the proposed amendment to claim 10 would overcome the Riedl et al. novelty reference.

Claims 1-3, 5-10, 12-47 and 85-88 are pending. Claims 1-3, 5, 46 and 47 are allowed. Proposed amendments to claims 10 and 88 are set forth herein.

Objection to the specification:

The disclosure is objected to because it recites the phrase "the conventional deoxynucleotides dATP, dCTP, dGTP and TTP" on page 23, line 5. The Office Action states that it is believed that applicants intended that "TTP' be "dTTP." The Office Action states that the previous argument, in which it was stated that TTP and dTTP are alternative ways of referring to the same molecule, and in which the Sigma catalog listing of the compound "Thymidine 5'-triphosphate" shows that it is referred to as either "TTP" or "dTTP", is not persuasive because "Thymidine 5'-triphosphate" is not a deoxynucleotide. The Office Action cites Stryer's biochemistry text, which lists deoxyadenosine, deoxyguanosine, **deoxythymidine** and deoxycytidine as the four nucleoside units in DNA.

Applicants submit, as stated in the previous response, that TTP and dTTP are alternative ways of referring to deoxythymidine triphosphate. Applicants note that the Stryer text cited by the Office Action does not mention the abbreviated term for deoxythymidine triphosphate. Applicants submit that reference to deoxythymidine triphosphate by the abbreviation dTTP is technically inaccurate, as this abbreviation actually refers to the dideoxy form of the nucleotide. Applicants refer the Examiner to Exhibits A and B, submitted herewith. Exhibit A is a photocopy of the listing for 5'-Deoxythymidine and 3'-Deoxythymidine 5'-triphosphate

trilithium salt in the 2002-2003 Sigma Catalog, page 641. There it is shown that the compound referred to as 5'-Deoxythymidine is "2',5'-**dideoxy**thymidine," and that 3'-Deoxythymidine 5'-triphosphate is referred to as "ddTTP-Li_3; ddTTP; dTTP; 2',3'-dideoxythymidine 5'-triphosphate." That is, 5'-Deoxythymidine and 3'-deoxythymidine 5'-triphosphate actually refer to the **dideoxy** form of the nucleotide. Applicants note that there is no other deoxythymidine triphosphate listed in the catalog.

Exhibit B is a photocopy from "The Encyclopedia Of Molecular Biology," J. Kendrew, ed., Blackwell Science, Ltd. (1994), page 762, in which Table N7 describes the nomenclature for the nucleosides and nucleotides of the bases found in RNA and DNA. The Examiner's attention is directed to the listing for "Thymine," which states that the nucleoside is "Thymidine*" and the nucleotide is "Thymidine monophosphate (thymidylic acid) (TMP), TDP, TTP." The asterisk calls one's attention down to the footnote, which states "**Thymidine, thymidine monophosphate, etc. usually refer to the **deoxy**ribonucleoside and **deoxy**ribonucleotide*" (Emphasis added). Further, Applicants note that each of Adenine, Guanine, and Cytosine have listings in the table for both the ribo- and deoxyribo- forms, i.e., adenosine and deoxyadenosine, guanosine and deoxyguanosine, and cytidine and deoxycytidine, but that thymine has only the listing for thymidine. Applicants submit that this is because, as noted in the footnote, "thymidine, thymidine monophosphate, etc.," including thymidine triphosphate, usually refers to the **deoxy**ribonucleotide.

In view of the above, Applicants submit that the abbreviation TTP refers to the **conventional, deoxy**-form of the thymidine 5'-triphosphate, and not to the **dideoxy, non-conventional** form of the nucleotide, represented by the abbreviation dTTP. Applicants also note that TTP cannot be confused with a ribonucleotide because RNA does have T residues. Thymidine is replaced by uridine (U) in RNA, so there can be no such confusion through use of the abbreviation TTP. Because the evidence presented herein above demonstrates that the proper abbreviation for deoxythymidine triphosphate is TTP, and that "dTTP" actually refers to the **dideoxy** form of the molecule, Applicants respectfully request the withdrawal of the objection.

Rejection under 35 U.S.C. §112, first paragraph, enablement:

The previous rejection of claims 6-15 under 35 U.S.C. §112, first paragraph, for lack of enablement is maintained. The previous Office Action stated that the use of *Thermococcus* species JDF-3 is essential to the claimed invention and that a deposit of the organism is required to enable the claims. Applicants traversed this rejection on the basis that *Thermococcus* strain JDF-3 is not essential because the specification provides polynucleotide and amino acid sequences for wild type JDF-3 polymerase and describes sites and substitutions for mutants that satisfy the limitations of claims 6-15. The present Office Action acknowledges that the specification does teach the *Thermococcus* strain JDF-3 polynucleotide and amino acid sequences and some substitutions which are encompassed by the claims. However, the Office Action states that "the claims do not recite any specific polynucleotide or amino acid sequences and thus claims to a Family B DNA polymerase from *Thermococcus* species JDF-3 require the *Thermococcus* species JDF-3." Applicants respectfully disagree.

Applicants submit that the specification consistently refers to the claimed Family B DNA polymerase from *Thermococcus* species JDF-3 with respect to SEQ ID NO: 2. As such, the provision of SEQ ID NO: 2 provides a literal boundary for what is meant by "Family B DNA polymerase from *Thermococcus* species JDF-3," and all that is needed to enable one of skill in the art to practice the invention is the sequence of SEQ ID NO: 2 itself. Support within the specification for the fact that the claimed *Thermococcus* species JDF-3 Family B DNA polymerases are properly regarded with respect to SEQ ID NO: 2 is provided as follows.

First, Applicants submit that the specification broadly describes the invention as being "based on the discovery of Family B DNA polymerases that bear one or more genetic alterations resulting in reduced discrimination against non-conventional nucleotides relative to their unmodified wild-type forms" (page 33, lines 1-3). However, claim 6 and its dependents are expressly limited to "an isolated Family B DNA polymerase from *Thermococcus* species JDF-3." The only Family B DNA polymerases from *Thermococcus* species JDF-3 reported in the specification are that of SEQ ID NO: 2 and mutants with amino acid substitutions stated relative to SEQ ID NO: 2. Thus, it is clear that the term "Family B DNA polymerase from *Thermococcus* species JDF-3" is used with respect to a polymerase of SEQ ID NO: 2 or a mutant thereof.

Further, the specification states "The polymerase from JDF-3 was chosen due to superior processivity, polymerization rate and ddNTP incorporation relative to the Family B DNA polymerase from Pyrococcus furiosus (Pfu)" (page 51, lines 15-17). Elsewhere, the specification states "To analyze JDF-3 mutant proteins, the DNA sequence encoding JDF-3 DNA polymerase was PCR amplified using primers {list of primer sequence omitted}" (page 57, lines 3-6). Applicants note that "The polymerase from JDF-3" and "the DNA sequence encoding JDF-3 DNA polymerase" as used in these passages are singular with regard to the polymerase. As noted above, the only Family B DNA polymerase from *Thermococcus* species JDF-3 provided in the specification is that of SEQ ID NO: 2 and mutants thereof. Thus, the specification makes it clear that mutants of the Family B DNA polymerase from *Thermococcus* species JDF-3 described are always referred to with respect to SEQ ID NO: 2.

Because the Family B DNA polymerase from *Thermococcus* species JDF-3 is consistently referred to with respect to SEQ ID NO: 2, one of skill in the art need only have available SEQ ID NO: 2 in order to generate Family B DNA polymerases from *Thermococcus* JDF-3 that fall within the claims. As such, Applicants submit that the law does not require a deposit of *Thermococcus* species JDF-3 to enable claim 6 or its dependents. Applicants respectfully request the withdrawal of this enablement rejection of claims 6 and its dependents.

Rejection under 35 U.S.C. §112, first paragraph, written description:

The previous rejection of claims 6-45 under 35 U.S.C. §112, first paragraph for lack of written description is maintained in the present Office Action. The Office Action states that "while Applicants have described a number of additional Family B polymerases and cited references which compare the sequences of many of these polymerases, Applicants have not described mutations which result in the desired polymerase properties in addition to those referred to in the previous Office Action." The Office Action further states that "the specification only provides the representative species encompassed by these claims, wherein said mutant polymerase is from *Thermococcus* species JDF-3 and the mutation is selected from the group consisting of mutations at residues: S345, P410, D141, E143, A485 and L408, of SEQ ID NO: 2." The Office Action continues:

> "While it is admitted that Applicants disclose a number of mutations, these are not representative of the genus of mutations claimed which encompasses any and all mutations of any Family B or *Thermococcus* species JDF-3 DNA polymerase which results in a decrease in 3' to 5' exonuclease activity or a reduction in discrimination against non-conventional nucleotides."

The Office Action concludes that "Given this lack of additional representative species encompassed by the claims, Applicants have failed to sufficiently describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicants were in possession of the claimed invention." Applicants respectfully disagree.

Under the Guidelines for Examination of Patent Applications Under the 35 U.S.C. §112, First Paragraph "Written Description" Requirement, the written description requirement for a genus claim may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics sufficient to show applicant was in possession of the claimed genus.

With respect to 3' to 5' exonuclease deficiency as recited in claim 6, Applicants submit that the specification describes six different individual mutants of *Thermococcus* species JDF-3 Family B DNA polymerase that target a region correlated with 3' to 5' Exo activity in related polymerases. These include D141A, D141N, D141S, D141T, D141E and E143A. The specification also describes the double mutant D141A + E143A, for a total of 7 mutants targeting the 3' to 5' Exo activity. Three of the single mutants and the double mutant exhibited dramatically reduced 3' to 5' Exo activity. Thus, the specification provides an actual reduction to practice through four working examples of different mutants that fall within the claim requiring 3' to 5' Exonuclease deficiency.

The Office Action states that the examples of species cited in the previous response "are still insufficient to adequately describe the claimed genuses drawn to all possible Family B DNA polymerases from *Thermococcus* species JDF-3 that are 3' to 5' exonuclease deficient." However, in addition to the working examples of Family B DNA polymerases from

Thermococcus species JDF-3 that are 3' to 5' exonuclease deficient, the specification provides a correlation between structure and function with regard to the 3' to 5' Exo activity. On page 56, the specification states:

> "The 3' –5' exonuclease activity associated with proofreading DNA polymerases can be reduced or abolished by mutagenesis. *Sequence comparisons have identified three conserved motifs, (exo I, II, III) in the 3' to 5' exonuclease domain of DNA polymerases* (reviewed by V. Derbyshire, J.K. Pinsonneault, and C.M. Joyce, Methods Enzymol. 262, 363 (1995)). *Replacement of any of the conserved aspartic or glutamic acid residues with alanine has been shown to abolish the exonuclease activity of numerous DNA polymerases*, including archaeal DNA polymerases such as Vent [citation omitted] and Pfu [citation omitted]. *Conservative substitutions lead to reduced exonuclease activity*, as shown for mutants of the archaeal 9°N-7 DNA polymerase [citation omitted]."

Thus, the specification specifically describes four mutants of the *Thermococcus* species JDF-3 Family B DNA polymerase that reduce 3' to 5' Exo activity, and also provides description of the structure/function relationship not only between the conserved structural motifs (i.e., exo I, II and III) and Exo activity, but also the structure/function relationship between the conserved motifs and particular amino acid changes (i.e., replacement of glutamic acid or aspartic acid residues with alanine) one should make within those motifs. Applicants have therefore provided an actual reduction to practice of four individual embodiments, plus functional characteristics (i.e., reduced Exo activity) coupled with a known *and* disclosed correlation between function and structure. Applicants further submit a description of every possible site at which a mutation affecting 3' to 5' Exo activity might occur is not required. According to the Written Description Guidelines, "description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces."

Because the specification provides a number of mutants targeting 3' to 5' Exo activity, four of which functionally reduce that activity, along with a description of conserved structural regions involved in such functional activity and specific mutations one should make in those regions in order to reduce 3' to 5' Exo activity, applicants submit that the Written Description requirement is satisfied with regard to the 3' to 5' Exo deficiency.

With regard to claim 10, Applicants submit that the claim as amended by the amendment proposed herein satisfies the written description requirement. The claim according to the proposed amendment requires that the isolated Family B DNA polymerase having reduced discrimination against non-conventional nucleotides "has a mutation in the Region II consensus sequence DXXSLYPSII." This consensus sequence is present at amino acids 404 to 413 of the *Thermococcus* species JDF-3 Family B DNA polymerase of SEQ ID NO: 2. Tables V and VI on pages 74 and 75 of the specification describe the isolation and testing of 12 Family B DNA polymerase mutants with mutations within this consensus region. Specifically, Table VI describes the isolation of 7 mutants with a primary mutation at L408 (3 are L408H, 4 are L408F) and 4 mutants with a primary mutation at P410 (all P410 L). Table V describes an additional P410L mutant (mutant p11). Thus, the specification provides 12 mutants, representing 3 different mutations within the Region II consensus recited in claim 10 as amended. Each of these mutants has a reduced discrimination against non-conventional nucleotides.

In addition to the description of the mutants in Tables V and VI, the specification provides additional description of numerous double mutants (a total of over 60 mutants are described in the specification). Ten such double mutants comprise at least one mutation within the Region II consensus region. These include: L408H + A485T, L408F + A485T, and P410L + A485T described on page 15, lines 14-22; P410H + S345P and P410L + S345P on page 16, lines 1-3; L408H + V437 and L408H + L478 on page 17, lines 4-7; and A485T + Y409V, L408 mutation + Y409V and P410 mutation + Y409V. In all, five different amino acid mutations are described within the Region II consensus sequence recited in claim 10: L408H, L408F, P410L, P410H and P409V.

The specification addresses the function of the Region II consensus structure at page 52, where it states:

> "The domains of relevance in 17 of the 40 purified mutants were sequenced. Most randomly mutated clones contained more than one mutation in the regions sequenced but all mutants contained mutations at one of three sites. Mutations predicted to confer an enhanced ddNTP uptake phenotype were introduced into the progenitor exonuclease deficient DNA polymerase sequence by site-directed mutagenesis to eliminate ancillary mutations which were not expected to contribute to the improved dideoxynucleotide uptake phenotype.

> Sixteen of the seventeen JDF-3 DNA polymerase mutations were found in region II (motif A) on either side of the tyrosine in the consensus sequence 404 DxxSLYPSII 413. These mutations consisted of DFRSLY**L**SII (P410L), DFRS**H**YPSII (L408H) and DFRS**F**YPSII (L408F)." (page 52, lines 13-23; emphasis added)

This passage points out that the mutations expected to have an effect on improved dideoxynucleotide uptake were centered in the Region II consensus sequence recited in claim 10 as amended. That is, the mutations outside of this region were intentionally *eliminated* before testing for reduced discrimination. This description provides a further structure/function correlation between the Region II consensus DxxSLYPSII and nucleotide discrimination.

In addition to the description of specific mutants and mutations in the Family B DNA polymerase of *Thermococcus* species JDF-3 discussed above, the specification provides description of other Family B DNA polymerases mutated in the Region II consensus and the effects of these mutations on discrimination against non-conventional nucleotides at page 7, line 20 to page 9, line 19. In particular, in reference to Y409 of the consensus sequence, the specification states "Mutagenesis studies done in Family B DNA polymerases also implicate the containing the analogous Y in region II in dNTP incorporation and ribose selectivity" (see p. 8, lines 11-12). The specification then continues to describe additional Region II mutations in other Family B DNA polymerases including the human DNA polymerase α (mutation at the site corresponding to Y409), bacteriophage T4 DNA polymerase (two mutations at the site corresponding to L408), bacteriophage ϕ29 DNA polymerase (mutations at the sites corresponding to L408 and P410), and the archaeal Family B DNA polymerase from *Thermococcus litoralis* (VENT; three mutations at a site corresponding to Y409) (see p. 8, line 12 to page 9, line 19. These mutants, the mutations of which each fall within the Region II consensus DXXSLYPSII, are discussed with regard to the impact of the mutations on nucleotide discrimination. Thus, the specification makes it clear that Region II structure, which is defined by the consensus DXXSLYPSII, is important in the function of nucleotide discrimination by Family B DNA polymerases.

Given the description in the specification of the structure/function relationship between the Region II consensus sequence DXXSLYPSII and reduced discrimination against non-conventional nucleotides, *and* the numerous (*at least 22*, given 12 mutants in Tables V and VI

and 10 double mutants described on pages 15-17) single and double mutants described in the specification that have mutations falling within this consensus, Applicants submit that a representative number of reduced discrimination mutants in the region recited in claim 10 as amended have been described to show that Applicants were in possession of the claimed genus. That is, applicants have adequately described a representative number of species within the claimed genus of isolated recombinant Family B DNA polymerases having reduced discrimination against non-conventional nucleotides, wherein the DNA polymerase has a mutation in the Region II consensus sequence DXXSLYPSII.

In view of the above, Applicants respectfully request the withdrawal of the §112, first paragraph, written description rejection of claim 10 and claims dependent from it.

Rejection under 35 U.S.C. §112, second paragraph:

Claims 10, 14, 15, 16-45 and 85-88 are rejected under 35 U.S.C. §112, second paragraph as indefinite for use of the term "non-conventional nucleotides." Applicants previously traversed this rejection, pointing out that the term is defined in the specification at page 25 as referring to "a) a nucleotide that is not one of the four conventional deoxynucleotides dATP, dCTP, dGTP and TTP, recognized by and incorporated by a DNA polymerase." The Office Action notes that the definition further states that the term refers to "or d) a ribonucleotide (since they are normally not recognized or incorporated by DNA polymerases) and modified forms of a ribonucleotide." The Office Action states that "In response to Applicant's argument here and above and the confusion in the specifications reference to "TTP" as being a deoxynucleotide, this definition and claim remains unclear." Applicants respectfully disagree.

As discussed above and documented in the accompanying exhibits, Applicants submit that TTP is an accepted alternative, and arguably more correct, way of denoting deoxythymidine triphosphate. In view of the discussion and additional documentation provided herein, Applicants respectfully submit that the term "non-conventional nucleotide" is definite. Applicants also wish to point out that there should be no confusion over whether TTP represents a ribo-, rather than a deoxyribo- nucleotide, because it is well known that RNA incorporates Uridine in place of thymidine. That is, there is no TTP incorporation in RNA synthesis, so there should be no confusion over T possibly being a ribonucleotide. In view of this and the

documentation provided in Exhibits A and B, Applicants respectfully request the withdrawal of the rejection of claims 10, 14, 15, 16-45 and 85-88 over this term.

Claims 10 and 85-87 are rejected under 35 U.S.C. §112, second paragraph as indefinite for use of the term "Region II." The Office Action states that the metes and bounds of the amino acids of Region II are not clear. Applicants submit that the proposed amendment of claim 10, which requires the Family B DNA polymerase to have a mutation in the Region II consensus sequence DXXSLYPSII is sufficient to overcome this rejection. Support for the amendment is found at page 52, lines 19-20, which state the region II consensus sequence as DXXSLYPSII, noting its location as amino acids 404-413 in the JDF-3 Family B DNA polymerase. Applicants respectfully request the withdrawal of this rejection of claims 10 and 85-87.

Claim 88 is rejected under 35 U.S.C. §112, second paragraph as indefinite because it is unclear if Applicants' recitation of "JDF-3 DNA polymerase" is the same as a "Family B DNA polymerase from *Thermococcus* species JDF-3." Applicants submit that the proposed amendment of claim 88 to recite an "isolated recombinant Family B DNA polymerase from *Thermococcus* species JDF-3" is sufficient to overcome this rejection. In view of the proposed amendment, Applicants respectfully request the withdrawal of this rejection of claim 88.

Rejection under 35 U.S.C. §102(e):

Claims 10, 11, 14, 15 and 44 are rejected under 35 U.S.C. §102(e) as being anticipated by Riedl et al. Applicants had previously argued that Riedl et al. fails to teach mutations in Region II of the Family B DNA polymerase, as required by claim 10. The Office Action states that the "argument is not found persuasive because Applicants have not supported their assertions as to which region the mutations of Riedl et al fall into." Applicants respectfully disagree.

Applicants initially note that claim 11 was cancelled in the last Office Action response, so this rejection is moot with regard to that claim.

Further, Applicants submit that the proposed amendment to claim 10, which requires that the DNA polymerase has a mutation in the Region II consensus sequence DXXSLYPSII is sufficient to distinguish the claimed invention over Riedl et al. Applicants submit that Riedl et al. teaches only tyrosine substitution mutations of every amino acid in the sequence

^{488}ILANSF^{493} of T. barosii DNA polymerase and mutations in the Exo region comprising amino acids 141 and 143 of the polymerase (see column 9, lines 24-32 and column 1, lines 63 to 65). Applicants submit that none of these mutations are in the Region II consensus sequence DXXSLYPSII, as demonstrated below.

First, Applicants submit that the present specification states that the consensus element in Region **III** is KX_3NSXYG:

> "*Region III of the Family B polymerases* (also referred to as motif B) has also been demonstrated to play a role in nucleotide recognition. *This region*, which corresponds to AA 487 to 495 of JDF-3 Family B DNA polymerase, *has a consensus sequence* KX_3NSXYG (Jung et al., 1990, supra; Blasco et al., 1992, supra; Dong et al., 1993, J. Biol. Chem. 268:21163; Zhu et al., 1994, Biochem. Biophys. Acta 1219:260; Dong and Wang, 1995, J. Biol. Chem. 270:21563), and is functionally, but not structurally (Wang et al., 1997, supra), analogous to $KX_3(F/Y)GX_2YG$ in helix O of the Family A DNA polymerases." (page 9, line 20 to page 10, line 2; emphasis added)

Applicants submit that the sequence ^{488}ILANSF^{493} of the *T. barosii* polymerase mutated by Riedl et al. falls within the sequence ^{487}KILANSFYG^{495} (see Riedl et al. Sequence Listing, columns 13 and 14). This is an exact match of the KX_3NSXYG consensus set out in the present specification as quoted above. Therefore, the mutations taught by Riedl et al. at ^{488}ILANSF^{493} of the *T. barosii* polymerase were in Region III, and *not* in Region II.

Second, Applicants submit that *T. barosii* Family B DNA polymerase described in Riedl et al. has an exact match for the sequence DFRSLYPSII, located at amino acids 404-413 (see Riedl et al. Sequence Listing at columns 13 and 14). Applicants further submit that Riedl et al. does not teach mutations within this sequence or in the immediately surrounding regions. The only other mutations taught by Riedl et al., other than the Region III mutations described above (^{488}ILANSF^{493} tyrosine substitutions), are the Exo$^-$ mutations at amino acids 141 and 143 (see column 1, lines 63-65). The amino acid 141 and 143 mutations are located approximately 350 amino acids amino-terminal to the consensus DxxSLYPSII sequence in Region II of the *T. barosii* Family B DNA polymerase. Therefore, Applicants submit that the 141 and 143 mutations taught by Riedl et al. are not in Region II. In view of the above, Applicants submit that Riedl et al. does not teach mutation in Region II, as required by claim 10, and therefore

cannot anticipate that claim. Therefore, claim 10 and its dependents cannot be anticipated by the Riedl et al. reference.

Regarding claims 14 and 15, Applicants submit that Riedl et al. cannot anticipate the claims because they depend from claim 6, which is limited to an isolated recombinant Family B DNA polymerase from *Thermococcus* species JDF-3. The polymerase taught by Riedl et al. is from *Thermococcus barosii,* not *Thermococcus* species JDF-3. The reference cannot, therefore, anticipate claim 14 or claim 15 which depends from it.

Claim 44 depends from claim 10 or claim 16 and requires a further mutation at an amino acid of the polymerase corresponding to one of amino acids 483 to 496, inclusive, of SEQ ID NO: 2. As discussed above, claims dependent from claim 10 cannot be anticipated by Riedl et al. because the reference does not teach a Family B DNA polymerase that has a mutation in the Region II consensus sequence DXXSLYPSII. With regard to claim 44 as it depends from claim 16, applicants submit that claim 16 requires a mutation at a site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408, S345 or P410 of SEQ ID NO: 2. As discussed above, the Riedl et al. reference teaches mutations only at ^{488}ILANSF^{493} of *T. barosii* DNA polymerase and in the Exo region comprising amino acids 141 and 143 of the polymerase. Applicants submit that none of these mutations taught by Riedl et al. correspond to A485, L408, S345 or P410 of SEQ ID NO: 2. As such, Riedl et al. cannot anticipate claim 16, nor can it anticipate claim 44 as it depends from claim 16.

In view of the above, Applicants submit that claims 10, 11, 14, 15 and 44 are novel over Riedl et al.. Applicants respectfully request the withdrawal of the §102(e) rejection of these claims over this reference.

In view of the preceding amendments and remarks, Applicants submit that all issues raised in the Office Action have been addresses herein. Applicants respectfully request reconsideration of the claims.

Respectfully submitted,

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Version of Amendments Marked to Show Changes:

In the claims:

Replace claims 10 and 88 with amended claims 10 and 88 as follows:

10. (Twice Amended) An isolated recombinant Family B DNA polymerase having reduced discrimination against non-conventional nucleotides, wherein said DNA polymerase has a mutation in [Region II] the Region II consensus sequence DXXSLYPSII.

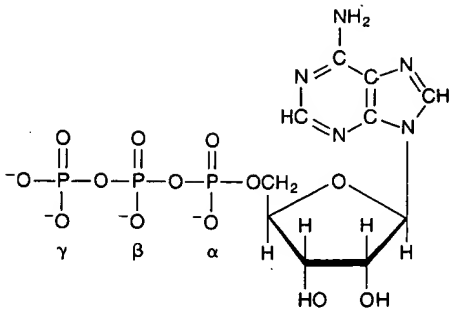
88. (Amended) An isolated recombinant Family B [JDF-3] DNA polymerase from *Thermococcus* species JDF-3 that comprises an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2.

				US $
D 2376	**Deoxyribonucleic acid methyl green** DNA-Methyl Green Substrate for Dnase	100 mg 500 mg	69.40 298.20	
D 3259	**Deoxyribonucleic acid-methyl green standardization sets** ***Components:*** DNA-Methyl Green, 100 mg DNase I, 5 vials	1 set	108.15	
	Deoxyribonucleic acid, Plasmid (pBR 325 Plasmid) from Escherichia coli HB101 See: Bacterial *Page 2155*			
	Deoxyribonucleic Acid, Sexed Female from human chorionic membrane See: Deoxyribonucleic acid, single stranded *Page 641*			
	Deoxyribonucleic acid, single stranded			
	Deoxyribonucleic acid, single stranded from salmon testes *[9007-49-2]* For use as a blocking agent in Northern and Southern blotting. **Ref.:** 1. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual* , Cold Spring Harbor, NY (1989), 9.48-9.49, 13.15 2. Silhavy, T., et al., *Experiments with Gene Fusions* , Cold Spring Harbor, NY (1984), 195			
D 7656 -0°C DRY ICE	**For Hybridization** This DNA is phenol-chloroform extracted, ethanol precipitated, and sonicated to produce single-stranded fragments which comigrate with the 587 and 831 base pair marker fragments. nucleic acid hybridization tested	1 mL 5 × 1 mL	51.75 126.90	
D 9156 -0°C DRY ICE	**For Hybridization** This DNA is ethanol precipitated and sonicated to produce single-stranded fragments which comigrate with the 587 and 831 base pair marker fragments.	1 mL 5 × 1 mL 5 mL	37.85 89.65 84.40	
D 8899 -0°C	**Deoxyribonucleic acid, single stranded from calf thymus** DNA single stranded *[91080-16-9]* **Lyophilized powder** Calf thymus DNA is exceptionally useful as a substrate for DNA polymerase assays, in the amplification of very long fragments and as a carrier DNA for precipitations. • Activated • No nuclease activity detected after 16h at 37°C incubation • High quality template DNA mol wt approx. 50 kb Lyophilized powder containing Trizma™ buffer salts Prepared by a modification of the method of Alberts and Herrick from calf thymus DNA. One mg DNA is approx. 25 A_{260} units. Package size based on DNA ssDNA minimum 65% **Ref.:** Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual* , Cold Spring Harbor, NY (1989), E.5 **Ref.:** Alberts, B., and Herrick, G., DNA-cellulose chromatography *Meth. Enzymol.* **21**, 198-217 (1971)	1 mg 5 mg 5 × 5 mg	17.40 58.80 260.10	
D 3287 -0°C	**Deoxyribonucleic acid, single stranded from human placenta** Deoxyribonucleic Acid, Sexed Female; from human chorionic membrane *[9007-49-2]* **For Hybridization** Fragments mol wt >20 kb (average length) This DNA is phenol-chloroform extracted, ethanol precipitated, and sonicated to produce single-stranded fragments which comigrate with the 587 and 831 base pair marker fragments. **Ref.:** 1. Sambrook, J., et al., *Molecular Cloning: A Laboratory Manual* , Cold Spring Harbor, NY (1989), 9.48-9.49, 13.15 2. Silhavy, T., et al., *Experiments with Gene Fusions* , Cold Spring Harbor, NY (1984), 195 S: 22-24/25	1 mL 5 × 1 mL	73.80 186.20	
D 8515 -0°C	**Deoxyribonucleic Acid–Cellulose** **Double-stranded from calf thymus DNA** DNA-Cellulose **Lyophilized powder** May contain a trace of Tris and EDTA. Extent of labeling 3-8 mg double-stranded calf thymus DNA per g **Ref.:** Alberts, B. and Herrick, G., DNA-cellulose chromatography *Meth. Enzymol.* **21**, 198-217 (1971)	1 g 5 g 10 g	36.20 117.40 210.40	
	Deoxyribonucleotides See: PCR *Page 1605*			
D 2751 2-8°C	**2-Deoxy-D-ribose** D-erythro-2-Deoxypentose; 2-Deoxy-D-erythropentose; Thyminose; 2-Deoxy-D-arabinose *[533-67-5]* $C_5H_{10}O_4$ FW 134.1 **minimum 99%**	5 g 25 g	48.85 149.05	
D 5899 2-8°C	**cell culture tested**	1 g	16.95	
D 7415 2-8°C	**2-Deoxy-L-ribose** *[18546-37-7]* $C_5H_{10}O_4$ FW 134.1	50 mg 250 mg	29.90 98.65	
D 6539 -0°C	**2-Deoxy-α-D-ribose 1-phosphate bis(cyclohexylammonium) salt** *[102783-28-8]* $C_5H_{11}O_7P \cdot 2C_6H_{13}N$ FW 412.5 **approx. 95%** Enzymatically prepared	5 mg 25 mg 100 mg 250 mg	78.75 261.75 726.35 1452.25	
D 3126 -0°C	**2-Deoxyribose 5-phosphate sodium salt** *[102916-66-5]* $C_5H_{11}O_7P$ FW 214.1 **95-98%** R: 23/24/25-36/37/38 S: 53-22-26-36-45	25 mg 100 mg	36.35 113.95	
	2-Deoxy-2-sulfamino-D-glucopyranose See: D-Glucosamine 2-sulfate *Page 947*			
	2-Deoxy-2-sulfamino-D-glucopyranose 3-sulfate See: D-Glucosamine 2,3-disulfate *Page 947*			
	11-Deoxytetrahydrocortisol See: 5β-Pregnane-3α,17α,21-triol-20-one *Page 1750*			
D 2640 -0°C	**5′-Deoxythymidine** 2′,5′-Dideoxythymidine *[3458-14-8]* $C_{10}H_{14}N_2O_4$ FW 226.2	10 mg	40.40	
	3′-Deoxythymidine 5′-triphosphate trilithium salt ddTTP-Li$_3$; ddTTP; dTTP; 2′,3′-Dideoxythymidine 5′-triphosphate *[93939-78-7]* $C_{10}H_{17}N_2O_{13}P_3$ FW 484.0 Inhibitor of DNA polymerase I - catalyzed chain elongation **Ref.:** Sanger, F., et al., *Proc. Natl. Acad. Sci. USA* **74**, 5463 (1977)			

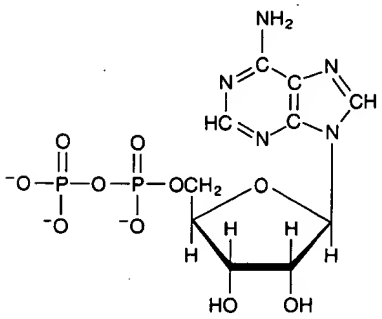
D

Deox

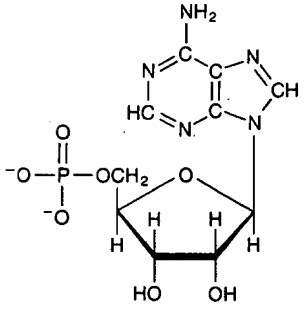
EXHIBIT B



Adenosine triphosphate (ATP)



Adenosine diphosphate (ADP)



Adenosine monophosphate (AMP)

Fig. N42 The ribonucleotides AMP, ADP, and ATP.

formed from the corresponding ribonucleotide by reduction of the 2′-OH on ribose catalysed by ribonucleotide reductase.

Nucleotides are involved in some way in most biological processes. Nucleoside triphosphates are the precursors for DNA and RNA synthesis, and nucleotides are activated intermediates in many reactions (*see* S-ADENOSYLMETHIONINE; SUGAR NUCLEOTIDES). ATP is the universal energy currency of living cells. Most cellular processes are powered directly or indirectly by the hydrolysis of ATP to ADP or AMP. The Gibbs energy of hydrolysis of the phosphoanhydride bonds ($\Delta G^{\circ\prime} = -30.5$ kJ mol^{-1}) is more negative than that of ordinary phosphate esters. The active form of ATP is usually complexed in the cell with Mg^{2+} (MgATP) or Mn^{2+}.

The adenine nucleotide derivatives (NAD, NADP, FAD, FMN, CoA) are vital cofactors in many metabolic processes, with the reduced forms of NAD and NADP (NADH and NADPH) being important sources of reducing power in cells. Guanine nucleotides are involved in the transduction of signals from many receptors (*see* G PROTEIN-COUPLED RECEPTORS; GTP-BINDING PROTEINS) and in PROTEIN SYNTHESIS. The cyclic nucleotides CYCLIC AMP and CYCLIC GMP act as second messengers (*see* SECOND MESSENGER PATHWAYS).

The importance of ribonucleotides as energy sources and cofactors in cellular processes may reflect their vital role in the evolution of life through the postulated RNA WORLD (*see also* MOLECULAR EVOLUTION).

Table N7 Nomenclature for the nucleosides and nucleotides of the bases found in RNA and DNA

Base	Nucleoside	Nucleotide
Adenine	Adenosine	Adenosine monophosphate, (adenylic acid) (AMP) Adenosine diphosphate (ADP) Adenosine triphosphate (ATP)
	Deoxyadenosine	Deoxyadenosine monophosphate (dAMP), dADP, dATP
Guanine	Guanosine	Guanosine monophosphate (guanylic acid) (GMP), GDP, GTP
	Deoxyguanosine	dGMP, dGDP, dGTP
Cytosine	Cytidine	Cytidine monophosphate (cytidylic acid) (CMP), CDP, CTP
	Deoxycytidine	dCMP, dCDP, dCTP
Uracil	Uridine	Uridine monophosphate (uridylic acid) (UMP), UDP, UTP
Thymine	Thymidine*	Thymidine monophosphate (thymidylic acid) (TMP), TDP, TTP

*Thymidine, thymidine monophosphate, etc. usually refer to the deoxyribonucleoside and deoxyribonucleotide.

nucleosome The repeating subunit in the organization of eukaryotic CHROMATIN. It is formed by ~200 bp of DNA double helix wrapped around a core particle which is an octamer composed of two copies each of HISTONES H2A, H2B, H3, and H4 (Fig. N43). Nucleosomes are connected by linker DNA which is, in most species, associated with histone H1, and associate with their neighbours to form a helical fibre of ~30 nm diameter. *See also*: PROTEIN–NUCLEIC ACID INTERACTIONS.

nucleotide(s) Constituents of nucleic acids (*see* DNA; NUCLEIC ACID STRUCTURE; RNA) which also have many other roles in all cells. *See*: NUCLEOSIDES AND NUCLEOTIDES.

nucle tide excision r pair *See*: DNA REPAIR.

nucle tid pair *See*: BASE PAIR.